CXLIV. THE ASCORBIC ACID CONTENT OF CERTAIN CITROUS FRUITS AND SOME MANUFACTURED CITROUS PRODUCTS.

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THE identification of vitamin C with ascorbic acid has evoked a number of papers in which titration methods for determination of the acid have been used to estimate the amount of vitamin C in natural products. Of particular interest in this respect are the papers concerned with the juice of citrus fruits [Bennett and Tarbert, 1933; Nelson and Mottern, 1933; Harris and Ray, 1933, 2; and Bessey and King, 1933], because of the general use and the consequent nutritive importance of these juices, because of the extent to which they enter into certain manufactured products, and also because the international standard preparation of vitamin C has been defined as the fresh juice of the lemon, *Citrus limonum*.

Our own attention was drawn to this problem somewhat indirectly; our results show general confirmation of the work of the investigators cited and certain other points that seem to us of special interest.

Ascorbic acid was determined by us as $C_6H_8O_6$, molecular weight 176, mainly with 2:6-dichlorophenolindophenol, by a method partly that of Harris and Ray [1933, 1] and partly that of Tillmans, whose method Harris and Ray modified. A standard solution of the dyestuff was run into the solution to be titrated; where this was a natural fruit juice, no addition was made to it, but solutions made by the extraction of portions of whole fruit were brought to the necessary degree of acidity with acetic acid. Originally we used a solution containing 0.1 g. of the dye per 100 ml., but later found that a 0.05 % solution, as suggested by Bessey and King [1933], gave equally good end-points with larger titres, and we therefore adopted it. 3 ml. portions of the juices were taken for the titration. The dye solution was standardised with ascorbic acid, the purity of which was itself checked by titration with standard iodine solution.

We also made use in some instances of titration with *N*/100 iodine solution. This was done for two reasons. In the first place, it was hoped that it might help to establish the presence of reducing substances other than ascorbic acid, since it is recognised that iodine is a less specific oxidising agent than 2:6-dichlorophenolindophenol; in the second place, we had been given, through the courtesy of Dr W. E. Baier, a number of results obtained by him and his colleagues in the laboratories of the California Fruit Growers' Exchange, where the iodine titration method had been used to follow through its various stages the large scale concentration of orange juice. Moreover, Dr Baier and his colleagues have themselves compared the two methods, and this has also been done by Bessey and King [1933]; it seemed therefore desirable to discover whether we would obtain the same correspondences or differences as other investigators.

Where iodine was used, 5 ml. of the fruit juice, or appropriate quantities of other products diluted with water, were taken for the titration; a few drops of fresh starch solution were added, and the iodine solution was run in slowly until a permanent blue colour was obtained. The end-point was fairly good, provided sufficient time was allowed for the titration.

Reproducibility of results.

We have investigated the reproducibility of results for both methods of titration. In most cases pairs of observations were made by two different observers; 33 pairs of determinations with 2:6-dichlorophenolindophenol gave a maximum variation between two observers of 0.02 mg. per ml. of juice (4 % of the mean value) and an average difference of 0.005 mg. per ml. (1 % of the mean value); the corresponding figures for 12 pairs of iodine determinations were 0.03 mg. (6 %) and 0.007 mg. (1.5 %). These figures apply to fresh fruit juices, but quite reasonably good agreement was also found for pairs of results obtained by either method on fruit extracts and manufactured products. It appears from these results that the 2:6-dichlorophenolindophenol method, which we agree with Harris and Ray in considering the most specific chemical test available for ascorbic acid, also gives slightly but appreciably more reproducible results than the iodine method.

Comparison of methods.

On comparing the two methods, it was found that orange and tangerine juices gave by the iodine method results that were, on the average, 1.5 % higher than those obtained with the dye, though in a number of instances the iodine results were the lower, the mean difference between all determinations by the two methods being, neglecting signs, 3.5 % of the mean value. Somewhat to our surprise, the values by the dye method for lemons were higher in every case than the values by the iodine method, the average difference being 2.5 % of the mean value. This is in direct contradiction to the results reported by Bessey and King [1933], who found a significant difference for oranges, the iodine results being higher, but no significant difference for lemons. As, however, comparisons were only made by us in quite a limited number of instances, we are not prepared to say more than that we think the matter requires further investigation. We are informed by Dr Baier (private communication) that he has found the results obtained on Californian Valencia oranges to be always higher by the iodine method than by the dye method.

CITROUS FRUIT JUICES.

In Table I are summarised the results obtained by us on three different species of the genus *Citrus*. The origin of each group was taken, as far as possible, from the papers in which the fruits were wrapped; the fruits themselves were all purchased from reputable dealers, but it is, in our opinion, desirable that an investigation of this sort should be carried out on a much larger scale by those engaged in research at the actual fruit-growing centres, not only because the origin and conditions of growth of the fruit can then be confidently stated, but also because the use of large numbers of fruits would enable the results to receive the necessary statistical treatment.

In spite of the obvious handicaps attached to *ad hoc* investigations of the kind conducted by us, we think the results are self-consistent and lead to certain definite conclusions, which are discussed below.

Table I.

Nature of fruit and date of purchase	Source of fruit	No. of samples	mg. ascorbic acid per ml. juice		ml. of juice per fruit		mg. ascorbic acid in juice of one fruit	
			Range of values	Mean value	Range of values	Mean value	Range of values	Mean value
Sweet orange (8. xii. 33)	Spain, Almeria	4	0.89	0.89	100-110	105	89-98	93
Sweet orange (seedless) (21. xii. 33)	Spain, Gandia	4	0.55-0.66	0.62	65-95	79	44-62	49
Sweet orange (8. xii. 33)	Palestine, Jaffa	4	0.53-0.63	0.58	110-125	116	61-72	67
Sweet orange (8. xii. 33)	Brazil	2	0.57-0.59	0.58	70	70	40-41	41
Sweet orange (9. xii. 33)	South Africa	4	0.34-0.68	0.57	90-115	107	39-74	60
Sweet orange (seedless) (8. xii. 33)	Spain, Tabernas	3	0.50-0.59	0.54	70-75	72	35-44	39
Sweet orange (8. xii. 33)	Brazil	4	0.40-0.42	0.40	80-90	85	34-36	35
Bitter orange (21. xii. 33)	Spain, Seville	6	0.36-0.56	0.43	25-35	31	10-17	14
Bitter orange (30. i. 34)	Spain, Seville	8	0.22-0.46	0.33	15-40	25	4-18	9
All oranges		39	0.22-0.89	0.51	15-125	67	4-98	39
Tangerine (8. xii. 33)	Algeria	2	0.73-0.78	0.75	35-45	40	27-33	30
Tangerine (8. xii. 33)	Spain	4	0.31-0.36	0.33	30-40	35	9-13	12
Tangerine (2. i. 34)	Jamaica	3	0.10-0.20	0.15	30-36	32	3-7	5
All tangerines		9	0.10-0.78	0.37	30-45	37	3-33	13
Lemon (8. xii. 33)	Spain, Malaga	3	0.62-0.73	0.68	45-60	50	33-37	35
Lemon (8. xii. 33)	Sicily, Messina	4	0.62-0.77	0.68	30-35	32	20-23	22
Lemon (8. xii. 33)	Italy	4	0.54-0.78	0.66	35-40	37	19-31	25
Lemon (21. xii. 33)	Cape Colony	4	0.47-0.62	0.54	30-40	35	17-21	19
All lemons		15	0.47-0.73	0.64	30-60	38	17-37	24

The figures in Table I show a very considerable range of variation for all three species of fruit. Although the range is greater for oranges than for lemons, we think this should be possibly attributed to the fact that we examined a greater number and more types of oranges than of lemons; Harris and Ray [1933, 2], who examined more lemons than oranges found a greater variation in the former than in the latter.

Stability of ascorbic acid in citrous fruits.

It seemed to us possible that there might be a loss of ascorbic acid from the plucked fruit, and that the difference between the figures of Harris and Ray [1933, 2] and our own might be due to this loss, especially since Bennett and Tarbert [1933] obtained, on the whole, higher values for the juice of freshly plucked fruits. We therefore carried out some keeping tests, without any special precautions against loss, the fruit being left in the open laboratory for approximately 4 weeks. The results are shown in Table II.

Table II. *Stability of ascorbic acid in whole fruit.*

	As purchased		After storage for 1 month	
	No. of samples	mg. ascorbic acid per ml.	No. of samples	mg. ascorbic acid per ml.
Almeria oranges	4	0.89	2	0.71
Jaffa oranges	4	0.58	2	0.52
S. Africa oranges	4	0.57	2	0.53
Tabernas oranges	3	0.54	1	0.51
Brazil oranges	4	0.40	2	0.27
Seville oranges	6	0.43	3	0.37
Spanish tangerine oranges	4	0.33	2	0.19
Averages for seven types of orange	29	0.535	14	0.44
Malaga lemons	3	0.68	1	0.67
Italian lemons	4	0.66	2	0.54
Cape lemons	4	0.54	2	0.57
Averages for three types of lemon	11	0.63	5	0.59

Concentrated orange juice.

By the courtesy of Dr W. E. Baier, we have received figures for the ascorbic acid content, determined by means of both 2:6-dichlorophenolindophenol and iodine, of orange juice immediately after expression and of the corresponding final concentrate, indicating a very small loss of potency. Although it was not possible for us to get a complete biological comparison to confirm this result, we submitted a sample of the concentrated juice to the Nutrition Department of the Pharmacological Laboratories of the Pharmaceutical Society for assay by their routine method. It was there compared biologically with a sample of pure ascorbic acid. The following figures have been obtained for this sample.

						Ascorbic acid mg. per g.
A. California Fruit Growers' Exchange						
	By titration	...	...	...	...	2.54
	By calculation	...	...	...	...	2.65
B. Glaxo Research Laboratory						
	By titration (4. i. 34)	...	...	...	...	2.55
	By titration (20. iii. 34)	...	...	...	...	2.47
C. Pharmaceutical Society						
	By biological assay	...	...	...	...	2.65
	By titration	...	...	...	...	2.28

The "calculated" figures from Dr Baier's laboratory are based upon the figures obtained for the fresh juice and the concentration of the finished product, as calculated from the weights of the fresh and concentrated juices. The titration figures shown were all obtained by the indicator method, though Dr Baier's technique differs from ours in certain minor details.

Marmalade.

Table III shows the result of examining a number of commercial brands of marmalade, most of which were obtained on the open market and the rest from the manufacturer, and one sample of home-made marmalade, prepared according to a well-established recipe.

Table III. *Ascorbic acid content of marmalade.*

	Apparent content of peel	Ascorbic acid (mg. per g.)	
		Indicator method	Iodine method
A	Very high	0.14	—
B	High	0.11	—
C	Medium	0.085	—
D	"	0.085 (0.08)*	0.095
D (2nd sample)	"	0.07 (0.07)*	0.105 (0.105)*
E	High	0.075	—
F	"	0.07 (0.075)*	0.07
F (2nd sample)	"	0.07	0.07
G	Low	0.065	—
H	"	0.06	—
I (6 samples)	Jelly (orange)	<0.01	0.035 (0.035)*
J (6 samples)	Jelly (lemon)	<0.01	1 sample only
K (home-made)	Medium	<0.01	—

* Values in brackets by second observer.

The figures reveal at once some correlation between the apparent amount of peel present and the ascorbic acid content.

Attention may be directed to the negative results obtained with home-made marmalade, which had presumably been submitted to a lengthy process of boiling in an open vessel; in any case, the sample was approximately a year old when the test was carried out, and it is most improbable that the commercial samples had reached this age. On the other hand, the 12 samples I and J represented six different stages of maturation of the particular marmalades, and no trace of reducing power was found in any of the samples.

Orange marmalade in this country is usually made from bitter Seville oranges. We understand that quantities of fruit in the neighbourhood of 15–30 lbs. per 100 lbs. of marmalade are normally used; a simple calculation will show that only in the very richest Seville oranges does the juice contain sufficient ascorbic acid for the reducing power of the richer marmalades to be derivable from the juice alone. It seemed to us, therefore, desirable to make at any rate a preliminary survey of the distribution of vitamin C through the different parts of the orange.

Distribution of ascorbic acid in citrous fruits.

Five Seville oranges in all were examined, as well as single specimens of sweet orange, tangerine and lemon. Extracts from different portions of the outer yellow skin (flavedo), the inner thick white skin (albedo) and the pips and pith were titrated, and the results of these experiments are summarised in Table IV.

Table IV.

Ascorbic acid (mg. per g.).

Fruit	Flavedo	Albedo	Whole peel	Juice	Pips
Seville orange	2.2	0.72	1.9	0.46	<0.01
„	1.8	0.77	0.86 (0.89)*	0.22	<0.01
„	—	—	0.96	0.30	—
„	—	—	0.96	0.32	—
„	—	—	1.2	0.28	—
Sweet orange	1.3 (1.4)*	0.69 (0.68)*	1.01 (1.02)*	0.72	—
Tangerine	—	—	0.69	0.15	—
Lemon	2.1 (2.2)*	0.46 (0.51)*	1.0	1.0	—

Weights of portions.

Fruit	Flavedo	Albedo	Whole peel	Juice	Pips
	g.	g.	g.	ml.	g.
Seville orange (average)	10	31	41	25	30
Sweet orange	12	31	43	40	30
Tangerine	—	—	18	30	7
Lemon	—	—	20	30	20

Average distribution of ascorbic acid in whole orange (Seville) as % of total.

		%			%
Flavedo	...	40	Juice	...	15
Albedo	...	45	Pips	...	0

* Values in brackets by iodine titration.

DISCUSSION.

The scope of our investigation naturally does not allow us to correlate the variation in ascorbic acid content of citrous fruit juices with many factors that might conceivably have a determining influence.

We have been informed that there is a considerable variation in the content of fruit solids not only in different fruits from the same tree, which might be

correlated with different degrees of ripeness, but in average fruits from neighbouring trees of undoubted genetic identity, grown under identical conditions, and there is no *a priori* reason for assuming that these variations will not apply to the ascorbic acid content.

Comparison of our results with those of Bennett and Tarbert showed that their averages for both oranges and lemons are definitely higher than ours. On the other hand, Harris and Ray found a considerably lower average value for lemons than we did. Table V summarises the figures reported by five groups of investigators for the ascorbic acid content of orange and lemon juices.

Table V.

	Oranges					Lemons				
	Maximum group range	Maximum group variation as % of mean	Total range	Maximum variation as % of mean	General mean	Maximum group range	Maximum group variation as % of mean	Total range	Maximum variation as % of mean	General mean
Bennett and Tarbert	—	—	0.91–1.03	15	0.97	0.43–0.88	70	0.43–0.94	75	0.68
Bessey and King	—	—	—	—	0.60	—	—	—	—	0.57
Harris and Ray	0.45–0.76	45	0.48–0.91	67	0.63	—	—	0.34–0.69*	73	0.47
Baier†	—	—	0.38–0.53	33	0.45	—	—	—	—	—
Bacharach, Cook and Smith	0.34–0.68	67	0.22–0.89	130	0.51	0.54–0.78	38	0.47–0.78	50	0.63

* Omitting one extremely low value (0.18).

† Group values—all Valentias.

Possible causes of variation in the ascorbic acid content of orange juice are many; among them are climatic conditions, including seasonal and annual variations in sunshine, rainfall and humidity, variety of fruit, soil and other cultural conditions, size and maturity of individual fruits, method of ripening and so on.

We think the results obtained by us on fresh fruit juices establish fairly definitely the contention made by one of us recently [Bacharach, 1933] that there is a dangerous tendency to generalise on the basis of insufficient experimental evidence about the amount of a particular vitamin “naturally” present in a given foodstuff. We have obtained indications not only of a very considerable range of values of ascorbic acid in, for example, oranges of different variety and origin, but even of very marked variations between the amounts present in the juice of fruits taken at hazard from the same consignment. A certain amount of work on this subject has already been published, but it is clear that the field remains still largely virgin soil.

In contradistinction to the definite loss of ascorbic acid found by us in oranges that had been kept for 1 month, Bracewell and Zilva, using the biological test [1931], reported “no marked loss in the vitamin content [of Jaffa oranges] when the fruit is stored under ordinary conditions at about 15° for 2 months.” It is, however, doubtful whether the amount of change taking place under the conditions mentioned by them would have been detected by biological assay, unless they had been able to use very much larger numbers of animals for the comparison and had been able to compare the juice before and after storage with some stable standard preparation.

We think our figures give evidence of deterioration even in so short a time as 1 month, and it seems to us a matter of some interest to those concerned with dietetics or with the fruit trade to see to what extent this change is normal and inevitable, for how long it continues, and what are the resultant limiting values for ascorbic acid content in different citrus fruit juices. It will be remembered

that Harris and Ray [1933, 2] found an apparent increase of ascorbic acid on storing lemons and attributed it to desiccation. The two effects are clearly antagonistic and one might mask the other.

The literature contains various accounts of investigations into the antiscorbutic potency of preserved (canned), concentrated, and dried orange and lemon juices, but in many cases the material examined was prepared on a relatively small scale. Priston [1926] described the examination of a concentrated Californian orange juice; his results suggested that there had been little destruction of its vitamin C content during processing. There were, however, no comparisons possible between the actual potency of the fresh juice and that of the manufactured product.

Goss [1925] tested biologically a number of products prepared commercially, including a vacuum-concentrated lemon juice, dried whole orange, desiccated orange juice and clarified concentrated orange juice; of the last named he reported that it "retained the antiscorbutic factor to a great extent although there is a probability that the longer exposure to the air at higher temperatures lowered this value." He used fresh orange juice as a standard antiscorbutic for control; this may have differed somewhat in vitamin C content from the fresh juice that was concentrated.

The results obtained on the sample of concentrated Californian orange juice, assayed at the Pharmacological Laboratories of the Pharmaceutical Society, indicate the possibility of conserving vitamin C even in large scale manufacturing processes. The excellent agreement between the biological and chemical assays again points to the presence in orange juice of negligible amounts of non-antiscorbutic substances having a reducing action on the dyestuff and iodine. It may be noted that, from Dr Baier's figures for the ascorbic acid contents of the original and the concentrated juice, and the weight concentration effected, it can be calculated that about 95 % of the original ascorbic acid is retained in the final product.

We feel justified in stating on the basis of the various results reported in this paper, that the preservation of vitamin C in products such as marmalade should offer no serious difficulties in properly organised and adequately controlled manufacture. By appropriate selection of fruit containing the right quantity and distribution of vitamin C, with special reference to the proportion of peel and juice to be included in the finished marmalade, the manufacturer should be able to produce a marmalade of standardised vitamin C content, and of any value required, within the limits permitted by the natural content of the fruit. The matter is by no means one of purely academic interest; there must be many households where practically the sole sources of vitamin C available to a growing family are potatoes and marmalade; variations in the antiscorbutic potency of the latter might have an appreciable effect on the vitamin C intake of the growing child.

As far as we have been able to discover, only one biological estimation of the vitamin C in marmalade has been reported in the literature. Delf [1921] fed to a scorbutic monkey a sample of orange marmalade, made without any special precautions to prevent destruction, except that the peel and solid parts had been boiled first and the juice added at the end. Delf reported that the marmalade had "a definite but small antiscorbutic value," about 1/10th that attributed by her to fresh orange juice. Delf, like ourselves, not unnaturally assumed that the vitamin C in marmalade was derived entirely from the orange juice used.

A comparison of the antiscorbutic potency of a sample of marmalade and the juice of fresh bitter oranges similar to those from which the marmalade had

been made was carried out in 1928 in the laboratories of the Pharmaceutical Society of Great Britain [Coward, 1934, private communication]. The marmalade was found to have about one-fourth to one-third of the vitamin C potency of the bitter orange juice. As the juice was known to constitute only about one-sixth of the finished marmalade, it was evident that some other constituent was contributing to the vitamin C content. The peel of the orange was suspected on the evidence of Hess [1916] as to the presence of vitamin C in orange rind, though no further experiments were carried out to try to confirm this.

Our results seem to us to indicate that a very considerable part of the vitamin C in marmalade, where it is present to an appreciable extent, is derived from the peel rather than from the juice. There is a marked correlation between the amount of peel apparently present in the marmalade, as far as can be judged by inspection, and the amount of ascorbic acid determined by titration, in spite of the probability that there must be numerous other independent variables operating to determine the final resultant ascorbic acid. In one or two instances we obtained higher results by the iodine titration than by the 2:6-dichlorophenolindophenol method. This might conceivably be due to the production during manufacture of sugar degradation products susceptible of oxidation by iodine solution but not reducing the dye under the conditions of the test. Nor must the possibility be overlooked that there may be produced sugar degradation products capable of reducing both reagents; there is no method known at present for establishing this point, save an extensive series of comparative chemical and biological tests. The recent work of Euler and Martius [1933] and others shows that physiologically inactive reducing substances can be prepared from simple sugars by heating under alkaline conditions, but we have found it also possible to obtain an increase in the reducing power of malt extract by simply heating it for $\frac{1}{2}$ to 2 hours at 100°; it is conceivable that traces of these substances might be produced during marmalade manufacture, even in the presence of natural fruit acids.

On the other hand, we have obtained some indication of the presence of inhibiting substances, or at any rate of technical difficulties in the estimation that result in the apparent presence of inhibiting substances. When pure ascorbic acid is added to orange or lemon juice, almost complete "recovery" is obtainable by either of the titration methods. This is not always the case if pure ascorbic acid is added to marmalade. Nevertheless, even bearing in mind the possible presence of reducing substances other than ascorbic acid, and of substances inhibiting the reduction of dyestuff and iodine, as well as the experimental errors inseparable from the titration of samples of marmalade, we are of opinion that the correlation between the apparent peel contents of marmalades and their reducing powers is too close to be other than significant.

Our results with the peel of citrous fruits might possibly have been attributed to the presence of reducing substances other than ascorbic acid, although the agreement between iodine and dye titrations is some evidence against such a view. We were therefore interested to find in the literature references to the antiscorbutic properties of orange peel. Hess and Fish [1914] report that "the juice of orange peel seems to serve the same purpose as the juice of the orange itself" in clinical experiments on infants. Hess and Unger [1918, 1] report that "a watery decoction of orange peel was able to protect against scurvy," and also [Hess and Unger, 1918, 2] that "orange peel possesses marked antiscorbutic potency, and withstands desiccation remarkably well." Willimott and Wokes [1926], however, found that an alcoholic extract of lemon flavedo was only feebly antiscorbutic; the conditions of preparation and storage of their

extract may well, however, have been such as to destroy any ascorbic acid present.

If our results lend themselves to the interpretation that there is a steadily falling concentration of vitamin C as one passes from the outside to the inside of the orange, it seems to us to open up some lines of investigation into the actual processes whereby ascorbic acid is synthesised in the plant. Is it possible that ascorbic acid is produced by some photosynthetic process involving the pigments themselves, and if so, which of the pigments in orange flavedo are responsible?

We shall content ourselves here with pointing to the unexpected similarity shown by the orange and the apple, since Bracewell *et al.* [1931] have fully demonstrated that the concentration of vitamin C in the tissue of Bramley's Seedling apples increases as the skin is approached from the core, and is more than six times as great in the peel as in the flesh near the core. The apparently very different morphology of the orange led us to assume, as we fancy has been assumed by other investigators, that the natural site of its vitamin C would be the juice, and that any present in the peel would be more or less adventitious. Our results suggest that this is very far from the truth.

The antiscorbutic activity of ascorbic acid has been generally accepted as about 16,000 units per g., but Key and Morgan [1933] in a recent paper reported only half this value and suggested that the discrepancy might be attributed to natural variations in lemon juice. The paper previously published by Bennett and Tarbert [1933] and the subsequent publication by Harris and Ray [1933, 2] lend some support to this suggestion, though Key and Morgan's value would necessitate that their lemon juice contained 1.35 mg. ascorbic acid per ml., a figure higher than has ever been reported by chemical determination, as indeed Harris and Ray themselves observe. The matter can never be finally settled since Key and Morgan carried out no chemical determinations of ascorbic acid on the lemon juice used for their comparisons. Qualitatively, however, we confirm the finding of Harris and Ray that fresh lemon juice as such, without further qualification, is obviously unfitted for use as an international standard preparation in vitamin testing.

The mean value found by us for lemon juice was 0.64 mg. per ml., which assigns to the international unit of vitamin C an ascorbic acid equivalent of 0.064 mg. This would give to ascorbic acid a value of nearly 16,000 international units per g. Since it is clear that our relatively small number of samples did not by any means cover the whole range of variation in lemon juices, no serious inaccuracy would be introduced into assays that have already been completed if the Permanent Commission for Biological Standardisation were to revise the international standard for vitamin C assay and assign to pure ascorbic acid an antiscorbutic activity of 10,000 or 20,000 international units per g.

SUMMARY.

Values are presented for the ascorbic acid content of the juice of individual fruits of oranges from nine different sources, tangerines from three sources and lemons from four sources. Determinations were made by titration with 2:6-dichlorophenolindophenol in acid solution, and in many cases with iodine also. These two methods gave results in good agreement.

Thirty-nine oranges gave extreme values of 0.22 and 0.89 mg. ascorbic acid per ml. of juice, with a mean of 0.51 mg. per ml. Nine tangerines gave extreme values of 0.10 and 0.78 mg. per ml., with a mean of 0.37 mg. per ml. Fifteen lemons gave extreme values of 0.47 and 0.73 mg. per ml., with a mean of 0.64 mg.

per ml. Considerable variations were often found even between fruits from the same consignment.

Storage of the fruit for 1 month caused on the average nearly 20 % loss of ascorbic acid in oranges, but only 6 % in lemons.

Examination of a concentrated orange juice by both chemical and biological methods showed that negligible loss of ascorbic acid had occurred during concentration and subsequent storage.

Eleven varieties of marmalade contained 0.06 to 0.14 mg. ascorbic acid per g. except for three varieties, which gave a negative test. The richer kinds were those apparently containing most peel.

In all types of citrus fruits examined, the flavedo was richest in reducing power, the albedo next richest; the juice was poorer in ascorbic acid than the peel, while the pips contained no demonstrable amount.

In view of the variable antiscorbutic potency of lemon juice, it is suggested that the international unit of vitamin C should be redefined as the vitamin C activity of some definite weight of ascorbic acid.

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